

Invasive plants affect arbuscular mycorrhizal fungi abundance and species richness as well as the performance of native plants grown in invaded soils

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Abstract We studied the effects of invasions by three plant species: *Reynoutria japonica*, *Rudbeckia laciniata*, and *Solidago gigantea*, on arbuscular mycorrhizal fungi (AMF) communities in habitats located within and outside river valleys. Arbuscular mycorrhizal colonization, AMF abundance and species richness in soils were assessed in adjacent plots with invaders and native vegetation. We also quantified the performance (expressed as shoot mass, chlorophyll fluorescence, and the concentration of elements in shoots) of two common, mycorrhizal native plants, *Plantago lanceolata* and *Trifolium repens*, grown in these soils. The invasions of *R. japonica*, *R. laciniata*, and *S. gigantea* influenced AMF communities compared to native vegetation, but the changes depended on the mycorrhizal status of invaders. The effects of non-mycorrhizal *R. japonica* were the most pronounced. Its invasion reduced AMF abundance and species richness. In the plots of both mycorrhizal plants, *R. laciniata* and *S. gigantea*, we observed decreased AMF species richness in comparison to native vegetation. The AMF community alterations could be due to (i) depletion of organic C inputs to AMF in the case of *R. japonica*, (ii) plant secondary metabolites that directly inhibit or selectively stimulate AMF species, or (iii) changes

in soil physicochemical properties induced by invasions. The effect of invasion on AMF abundance and species richness did not generally differ between valley and outside-valley habitats. The invasions affected photosynthetic performance and the concentrations of elements in the shoots of *P. lanceolata* or *T. repens*. However, the directions and magnitude of their response depended on both species identity and the mycorrhizal status of invaders.

Keywords Arbuscular mycorrhiza (AM) · *Plantago lanceolata* · *Reynoutria japonica* · *Rudbeckia laciniata* · *Solidago gigantea* · *Trifolium repens*

Introduction

Biological invasions, next to the habitat changes caused by human activity, are the second most important factor threatening biodiversity at the species, biotope, and landscape levels (Mirek 2010). The spread of alien plant species significantly affects abundance and diversity of soil microorganisms, including the most widespread and important plant symbionts, arbuscular mycorrhizal fungi (AMF) (Mummey and Rillig 2006; Callaway et al. 2008; Vogelsang and Bever 2009; Tanner and Gange 2013). These fungi colonize the roots of ca. 80 % of land plant species and have been found to increase nutrient acquisition, growth, and vitality of their hosts. They are also crucial for the protection of plants against biotic (pathogens) and abiotic (drought, heavy metal toxicity) stresses and have been found to determine plant community composition and function (Smith and Read 2008). The changes in AMF abundance and species richness caused by alien plants can thus be detrimental to native plant species and accelerate plant invasions (Reinhart and Callaway 2006; Shah et al. 2009).

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Although the impact of several alien plant species on AMF abundance and species richness has been studied recently (Liang et al. 2004; Zhang et al. 2010; Tanner and Gange 2013; Yang et al. 2014), the majority of plants from the transformer category, namely taxa with potentially most negative influence on the biodiversity of natural habitats (Tokarska-Guzik et al. 2012), have not been investigated so far in this context. Moreover, there is no doubt that invasive plants alter soil microbiological properties, but directions of the changes and their magnitude may differ between habitats. As it was underlined by Shah et al. (2009) and Lekberg et al. (2013), the comparisons of effects of multiple plant species invading different habitats have been neglected so far. Therefore, we investigated the effects of invasions of three transformers, namely *Reynoutria japonica* Houtt. (Polygonaceae), *Rudbeckia laciniata* L., and *Solidago gigantea* Aiton (Asteraceae), on AMF abundance and species richness in sites located within and outside river valleys. *Rudbeckia laciniata* and *S. gigantea* are plant species of American origin while *R. japonica* is native to Asia. They have spread in Europe and form extensive, almost monospecific patches with negative effects on biodiversity (Tokarska-Guzik et al. 2012). In Poland, these species were introduced as ornamental plants and escaped from cultivation into the wild. They invade a range of sites such as riparian zones around the standing waters or streams, floodplain woods, forest clearings, swamps, wastelands, grasslands, cultivated field margins, fallows, roadsides, and areas along railroad tracks (Dajdok and Pawlaczyk 2009; Tokarska-Guzik et al. 2012). *Rudbeckia laciniata* and *S. gigantea* form arbuscular mycorrhiza (AM), and *R. japonica* is a non-mycorrhizal species (Wang and Qiu 2006; Štajerová et al. 2009; Majewska et al. 2015). The impact of *R. laciniata* and *S. gigantea* invasions on AMF communities has not been studied so far, and in the case of *R. japonica*, such investigations have been conducted only once. Tanner and Gange (2013) showed the negative effect of *R. japonica* on AMF abundance in one location in the UK.

The aim of the study was to evaluate the impact of *R. japonica*, *R. laciniata*, and *S. gigantea* invasions on AMF abundance and species richness in sites located outside and within river valleys. We selected, in total, 48 independent sites of these three species to obtain general conclusions on the effects of their presence. Soil and root samples from the nearly monospecific patches of the invaders and native vegetation located in the vicinity were collected. The performance of native plants, *Plantago lanceolata* L. (Plantaginaceae) and *Trifolium repens* L. (Fabaceae), grown in these soils was also investigated in a laboratory experiment. Our hypothesis was that the direction of the changes would depend on mycorrhizal status of the investigated invasive plants. The presence of non-mycorrhizal *R. japonica* would decrease both AMF species richness and propagule abundance, and this would result in a decrease in the percentage colonization of AMF of the roots of

native plants grown in soils from under the invader. As a consequence, the native plants grown in such soils would have reduced performance (shoot mass, the concentration of elements in shoots, and photosynthetic parameters), compared to plants grown in soils from beneath native vegetation. Invasions of mycorrhizal *S. gigantea* and *R. laciniata* would have no effect on AMF abundance; however, given the existence of the selectivity and functional diversity in AM symbiosis (Helgason et al. 2002; Smith and Read 2008; Holland et al. 2014; Taylor et al. 2015), we also expected AMF species composition alterations in soils overgrown by these plants.

Materials and methods

Study region, plant and soil sampling

We investigated the effects of invasions of three vascular plant species of alien origin in Europe, *R. japonica* [= *Fallopia japonica* (Houtt.) Ronse Decr., = *Polygonum cuspidatum* Sieb. et Zucc.], *R. laciniata*, and *S. gigantea* (= *S. serotina* Aiton). Soil and root samples were collected from 23 to 27 August 2013 from non-forest habitats (fallow, meadows) in southern Poland. The study area covered ca. 7500 km². Forty-eight independent study sites located within (24 sites) and outside (24 sites) river valleys were selected. In the case of river valley sites, the samples were collected from up to 50 m from the riverbed, i.e., from areas defined as floodplains. We assumed that the within and outside river valley sites differ in terms of the presence of alluvial materials as well as microclimate. The invaded patches selected for the study were dense, almost monospecific stands (>90 % cover) of *R. japonica*, *R. laciniata*, or *S. gigantea*, while the adjacent patches of multi-species native vegetation were devoid of invasive species (control). The number of vascular plant species in the invaded and native vegetation plots ranged from 1 to 11 (3.5 on average) and from 4 to 21 (11 on average), respectively. *Reynoutria japonica* and *S. gigantea* valley sites were dominated by *Phalaris arundinacea* and *Rubus caesius*, followed by *Petasites hybridus*, *Calystegia sepium*, *Aegopodium podagraria*, and *Urtica dioica* (*R. japonica* valley sites) or *Cirsium arvense* and *Tanacetum vulgare* (*S. gigantea* valley sites). *Rudbeckia laciniata* valley sites were dominated by *C. arvense*, *Galeopsis speciosa*, *Dactylis glomerata*, and *Chaerophyllum aromaticum*. *Calamagrostis epigejos*, *Arrhenatherum elatius*, *C. arvense*, and *Equisetum arvense* dominated both *R. japonica* and *S. gigantea* outside-valley sites. These species were often accompanied by *R. caesius* (*R. japonica* outside-valley sites) or *Agropyron repens* and *T. vulgare* (*S. gigantea* outside-valley sites). The patches of native plants in *R. laciniata* outside-valley sites most often consisted of *A. elatius*, *C. arvense*, *Holcus lanatus*, and *Centaurea phrygia*. The nomenclature of plant species

follows Mirek et al. (2002). Soil chemical properties of the invader and native vegetation plots are presented in Table 1 (Stefanowicz et al., unpublished).

In the sites of each invasive plant species, soil and root samples were collected from 2×2 m plots in both invader and native vegetation patches. The contrasting (invader vs. native) plots were located as close as possible, a maximum of 15 m from each other, in order to avoid any differences in soil properties existing prior to invasions, and at least 3 m from the edge of a patch so as to minimize any effects of contrasting vegetation such as shading or litterfall. Three subsamples of topsoil (ca. $20 \times 20 \times 20$ cm, 8 l) with roots were randomly excavated using shovels from each individual plot. Soil organic horizon, consisting of partly decomposed litter, was removed and shoots were cut. Half of the volume of each subsample (ca. 4 l), obtained by cutting a cube parallel to the soil profile, was placed in a plastic bag and bulked to obtain one composite sample (ca. 12 l) per plot. The roots were manually cleaned of soil. In the case of each sample, the roots were randomly cut and placed in a plastic container to fill the entire volume (60 ml). They were preserved in 50 % ethanol in water. Sterile gloves were used for handling and processing each soil-root sample to avoid contamination. In total, 96 soil and 96 root samples were collected: 3 invasive plant species \times 2 invasive-native paired plots \times 2 habitats (within and outside valley) \times 8 replicates.

The roots were stained for the visualization of root-inhabiting fungi. The soils were used for the isolation of AMF spores, phospholipid fatty acid (PLFA) 16:1 ω 5 analysis of AMF abundance, and were also utilized in a laboratory experiment aimed at determining native plant performance in the invaded soils (see below).

Root staining and the assessment of fungal colonization

The roots collected from both plot types, which were preserved in ethanol, were washed in tap water to remove the remnants of soil. They were then stained according to Phillips and Hayman (1970) method, with minor modifications incorporated by Zubek et al. (2012). For each sample, 30 stained root fragments approximately 1 cm long were randomly chosen, mounted on slides in glycerol:lactic acid (1:1), and pressed using cover slides. The fungal colonization of roots was assessed using a Nikon Eclipse 80i microscope with Nomarski interference contrast. The degree of AMF colonization was determined following the calculation of mycorrhizal frequency ($F_{AMF}\%$), relative mycorrhizal root length ($M_{AMF}\%$), and relative arbuscular richness ($A_{AMF}\%$) in accordance with the method proposed by Trouvelot et al. (1986). An estimate of $F_{AMF}\%$ is given as the ratio between root fragments colonized by AMF mycelium and the total number of root fragments analyzed. Parameter $M_{AMF}\%$ is an estimate of the proportion of the root cortex that is mycorrhizal relative to

Table 1 Chemical properties of soils in invader (I) and native (N) vegetation plots in habitats within and outside river valleys (means \pm standard deviations, $N=8$)

Variable	<i>Reynoutria japonica</i>				<i>Rudbeckia laciniata</i>				<i>Solidago gigantea</i>			
	Outside-valley		Valley		Outside-valley		Valley		Outside-valley		Valley	
	N	I	N	I	N	I	N	I	N	I	N	I
pH H_2O	7.7 \pm 0.7	7.8 \pm 0.8	7.7 \pm 0.7	7.5 \pm 0.8	6.4 \pm 0.6	6.5 \pm 0.6	6.6 \pm 0.7	6.5 \pm 0.4	6.7 \pm 0.6	6.6 \pm 0.8	7.6 \pm 0.7	7.6 \pm 0.7
$C_{organic}$ (%)	2.8 \pm 1.9	2.4 \pm 1.3	1.5 \pm 0.7	1.6 \pm 1.0	2.0 \pm 0.6	1.9 \pm 0.4	2.6 \pm 0.4	2.6 \pm 1.1	2.3 \pm 0.9	2.2 \pm 0.7	2.2 \pm 0.8	2.3 \pm 0.8
N_{total} (g kg^{-1})	2.3 \pm 1.2	2.2 \pm 1.3	1.6 \pm 0.5	1.4 \pm 0.6	2.2 \pm 0.6	2.0 \pm 0.5	2.5 \pm 0.5	2.6 \pm 1.0	2.4 \pm 0.9	2.1 \pm 0.5	1.8 \pm 1.0	1.8 \pm 0.8
$N-NH_4^+$ (mg kg^{-1})	10.6 \pm 7.5	9.1 \pm 8.2	4.2 \pm 3.4	3.6 \pm 2.5	7.5 \pm 5.0	6.8 \pm 5.7	3.3 \pm 2.5	2.9 \pm 1.4	3.8 \pm 3.2	6.5 \pm 6.7	6.5 \pm 6.7	4.5 \pm 2.4
$N-NO_3^-$ (mg kg^{-1})	7.3 \pm 6.5	19.2 \pm 11.5	12.2 \pm 9.3	17.7 \pm 12.0	17.2 \pm 10.7	10.1 \pm 6.9	20.0 \pm 13.1	17.0 \pm 9.2	7.5 \pm 4.1	6.2 \pm 5.9	10.4 \pm 10.1	5.2 \pm 6.2
P_{total} (mg kg^{-1})	851 \pm 377	848 \pm 508	393 \pm 263	265 \pm 66	732 \pm 434	404 \pm 217	533 \pm 535	287 \pm 102	651 \pm 478	680 \pm 536	606 \pm 254	496 \pm 252
C:N	12 \pm 3	11 \pm 3	9 \pm 2	11 \pm 6	9 \pm 2	10 \pm 1	11 \pm 1	10 \pm 1	10 \pm 1	11 \pm 1	14 \pm 7	14 \pm 5
C:P	41 \pm 39	44 \pm 37	48 \pm 30	62 \pm 46	40 \pm 29	62 \pm 34	89 \pm 62	94 \pm 36	65 \pm 72	69 \pm 68	45 \pm 28	67 \pm 62

the whole analyzed root system. Arbuscule abundance ($A_{AMF}\%$) is an estimate of arbuscule richness in the whole analyzed root system (Trouvelot et al. 1986).

Other root-inhabiting fungi, such as dark septate endophytes and *Olpidium* spp., were observed during the examination of roots and thus their abundance was also assessed. Dark septate endophyte colonization was identified on the basis of melanized hyphae that colonize the cortical cells and intercellular regions of roots and facultatively form microsclerotia (Jumpponen 2001). Frequency of dark septate endophyte mycelia occurrence in roots ($F_{DSE}\%$) was estimated as detailed above for AMF. Similarly, the frequency of occurrence for resting sporangia of fungi from the genus *Olpidium* (Webster and Weber 2007) ($F_{Olp}\%$) was assessed.

AMF spore isolation and identification

Directly after the material collection, spores were isolated by centrifuging the soils in 50 % sucrose solution and filtering (Brundrett et al. 1996). The spores were transferred to Petri dishes, and their numbers in the tested soils were counted under dissecting microscope. All spores from each sample were then mounted on a slide in a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) and a mixture of PVLG/Melzer's reagent (4:1, v:v) (Omar et al. 1979). The identification of AMF spores was carried out using an Olympus BX51 light microscope following Błaszczowski (2012).

PLFA marker of AMF abundance in soils

Phospholipid fatty acid (PLFA) 16:1 ω 5 marker was utilized to assess the AMF abundance in soils (Olsson 1999). The analysis was performed according to Palojarvi (2006), with the exception of the lipid extraction, which followed Macnaughton et al. (1997). Lipids were extracted from freeze-dried soil samples with a mixture of methanol/chloroform/phosphate buffer (2:1:0.8, v:v:v) using accelerated solvent extractor ASE 200 (Dionex; two 15-min cycles, 80 °C, 1200 PSI). The lipids were separated into neutral-, glyco-, and phospholipids in Bakerbond silica gel SPE columns (500 mg, Baker) by eluting with chloroform, acetone, and methanol, respectively. The phospholipids were subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters were separated and identified using a Varian GC-MS system (Varian 3900 and Saturn 2100 T) and NIST library. The CP-Select CB for FAME (50 m \times 0.25 \times 0.39) column (Agilent Technologies) was used. Helium was used as a carrier gas, and injections were made in split mode (1:100). Methyl nonadecanoate (19:0; Fluka) was used as an internal standard. The identification and response factors of 16:1 ω 5 methyl ester were based on cis-11-hexadecenoic acid standards (Matreya LLC) after mild alkaline methanolysis.

Assessment of AMF colonization and performance of native plants in soils from under invader and native vegetation

In order to assess AMF colonization and native plant performance in the invaded soils, a laboratory experiment was conducted. Individual soil samples (450 ml of each) collected from 96 plots with invasive and native plant species were placed in 500-ml sterile plastic pots that were 9 cm wide and 12.5 cm high. Sterile gloves were used for processing each sample to avoid contamination. Two common, native to Europe, mycorrhizal model plant species were utilized in the experiment, namely *Plantago lanceolata* L. and *Trifolium repens* L. The seeds of these species were obtained from Herbador (Poznań, Poland) and Planta (Tarnów, Poland), respectively. Approximately ten seeds of these plants were seeded per pot. After 1 week, the plants were thinned out to obtain five individuals in each pot. In total, 192 cultures were established with *P. lanceolata* (96 pots) and *T. repens* (96 pots). All the cultures were kept in open Sigma-Aldrich sun bags, to avoid contamination between treatments, under plant cultivation room conditions at a temperature of 22 ± 2 °C. The following light regime was employed: 270–280 μ mol PAR photons \times m⁻² \times s⁻¹, 12/12 h. The pots were arranged in a completely random manner. The cultures were watered once a week using 35 ml of distilled water.

After 7 weeks of growth, chlorophyll *a* fluorescence measurements were conducted, and then the plants were harvested. The plants were rinsed with tap and deionized water. The bulked roots of each pot were stained in order to visualize AMF mycelia for the mycorrhizal colonization assessment. The shoots of each individual plant were dried at room temperature before determining their biomass. They were weighed using an electronic analytical balance (Radwag, WPA 60/c/1) with a level of precision of 0.0001 g. The shoots from each individual pot were bulked and analyzed for their element content.

Evaluation of photosynthetic performance

Chlorophyll *a* fluorescence transients OJIP were measured with a Handy PEA fluorimeter (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The measurements were conducted on fully expanded leaves, still attached to the plants, which were dark-adapted for 30 min prior to measuring. The measurements were carried out as described by Strasser et al. (2004) and Tsimilli-Michael and Strasser (2008). Five to ten measurements were performed on randomly chosen leaves of plants in each pot. The data from each individual pot were then averaged to finally obtain eight repetitions per treatment.

For each repetition sample (pot), the average OJIP fluorescence transients were analyzed according to the JIP test (Strasser et al. 2004), with “Biolyzer” software (Laboratory

of Bioenergetics, University of Geneva, Switzerland). The parameter chosen for presentation was performance index on absorption basis (PI_{ABS}), which evaluates overall photosynthetic performance (Tsimilli-Michael and Strasser 2008).

Measurement of element concentrations in shoots

The shoot samples of *P. lanceolata* and *T. repens* were dried at 80 °C and ground with a Pulverisette 14 variable-speed rotor mill (Fritsch, Germany). The samples were mineralized in a mixture of suprapure concentrated HNO_3 and $HClO_4$ (Merck) (4:1, v:v), using a hotplate (Foss Tecator Digestor Auto, Germany). The digestive was concentrated by evaporation to about 0.5 ml and then diluted with deionized water (17 mΩ) to a final volume of 10 ml. Total P was measured with the molybdenum-vanadate method on a Hach-Lange DR 3800 apparatus. Total N was determined according to the Kjeldahl method using a Kjeltac 2300 apparatus (Foss Tecator, Denmark). The concentrations of Cu, Zn, Ca, and Mg were determined using a Fast Sequential Atomic Absorption Spectrometer 280 (Varian, Australia). Accuracy was verified using reference international interlaboratory moss samples M2 (Steinnes et al. 1997).

Statistical analyses

Dependent variables were examined for normality and homogeneity of residuals (using the Shapiro-Wilk test and Levene's test, respectively) and then transformed with logarithmic or exponential functions to meet parametric assumptions. Split-plot ANOVA (with sites acting as blocks) was used to examine the effect of invasion (plots of native vegetation vs. plots overgrown by invasive plant species; within-block factor), the effect of site (*R. japonica* vs. *R. laciniata* vs. *S. gigantea* sites, including plots of both invader and native vegetation; between-block factor), the effect of habitat (within vs. outside river valleys; between-block factor) and the interactions of the above factors on the variables describing the abundance of AMF and DSE in the field, and the performance of native plants grown in soils from under invader and native vegetation. If the effect of invasion interacted with another effect, contrast analysis was used to compare the invader and native vegetation plots separately for each level of a given effect.

Among the analyzed variables were two representing the AMF species composition (DCA 1 and DCA 2). They were sample scores of the first two axes obtained using detrended correspondence analysis (DCA), which was performed on the AMF species dataset. This analysis excluded rare species (i.e., found in <5 % sample plots). Statistical analyses were performed with Statistica 9 (Statsoft, Tulsa, OK) and Canoco 5 (Ter Braak and Šmilauer 2012).

Results

Fungal root colonization of field-collected plants

Arbuscular mycorrhizal fungi

The effect of invasion on mycorrhizal colonization parameters depended on the invader species, as indicated by a significant site \times invasion interaction (Table 2). The level of arbuscular mycorrhizal fungi (AMF) colonization, represented by the three mycorrhizal parameters (F_{AMF} , M_{AMF} and A_{AMF}), was reduced in the *R. japonica* plots (to zero in habitats outside the river valleys), while raised in the *R. laciniata* and *S. gigantea* plots in relation to that associated with native vegetation. As the mycorrhizal parameters almost ideally correlated with each other (Pearson's r was from 0.95 to nearly 1), only the mycorrhizal frequency (F_{AMF}) is presented (Fig. 1a). The effect of invasion was slightly stronger in habitats outside the river valleys. This is evidenced by a significant invasion \times habitat interaction detected for relative arbuscular richness (A_{AMF}).

Dark septate endophytes and *Olpidium*

The percentage of root colonization by dark septate endophytes was low in all plots. Only single hyphae, accompanied sporadically by microsclerotia, were found in the outer cortex and rhizodermis. The dark septate endophyte colonization was influenced by the invader species in a similar manner as the AMF colonization (significant site \times invasion interaction; Table 2). The abundance of dark septate endophytes was reduced in the *R. japonica* plots, whereas it was increased in the *R. laciniata* plots in relation to that recorded in the adjacent plots with native vegetation (Fig. 1b). The effect of *S. gigantea* invasion on dark septate endophytes was insignificant.

The root systems in all invader plots were devoid of *Olpidium*. Their single sporangia were found inside root epidermal cells in only five samples of native vegetation (data not presented).

AMF abundance as revealed by PLFA analysis

The effect of invasion on the concentration of phospholipid fatty acid (PLFA) 16:1 ω 5 in the soil depended on the invader species (significant site \times invasion interaction; Table 2). The invasion of *R. japonica* decreased PLFA 16:1 ω 5 concentration. In the case of *R. laciniata* or *S. gigantea*, there were no significant differences in the concentration of PLFA 16:1 ω 5 in soils from adjacent plots with invasive and native plants (Fig. 1c).

Table 2 *F* values of split-plot ANOVA for the effects of site (*Reynoutria japonica* vs. *Rudbeckia laciniata* vs. *Solidago gigantea* sites), habitat (within vs. outside valleys), invasion (invader vs. native vegetation plots) and their interactions on arbuscular mycorrhizal fungi(AMF) and dark septate endophytes (DSE) abundance, and AMF species richness and composition, as well as the performance of native plants (*Plantago lanceolata*, *Trifolium repens*) grown in soils from under invader and native vegetation

Variable	Site	Habitat	Site × habitat	Invasion	Invasion × site	Invasion × habitat	Invasion × site × habitat
Fungal root colonization in invader and native vegetation plots							
F_{AMF} —mycorrhizal frequency	36.4***	3.6	0.0	5.0*	37.1***	3.9	0.2
M_{AMF} —relative mycorrhizal root length	33.4***	3.8	0.6	5.9*	33.9***	3.7	0.2
A_{AMF} —relative arbuscular richness	35.1***	4.4*	0.6	5.6*	33.4***	4.6*	0.1
F_{DSE} —frequency of DSE occurrence	15.6***	0.8	0.2	0.2	8.1**	1.7	0.8
Concentration of 16:1ω5 PLFA	2.6	3.4	1.2	0.0	7.3**	0.2	1.5
Number of AMF spores	7.1**	0.6	0.2	23.4***	13.4***	0.2	1.0
Number of AMF species (species richness)	6.4**	3.5	0.4	7.5**	0.6	3.6	0.0
AMF species composition—DCA 1 sample scores	0.2	1.2	0.5	2.7	0.3	0.2	0.0
AMF species composition—DCA 2 sample scores	8.1**	0.3	2.7	1.7	0.2	0.0	0.2
<i>Plantago lanceolata</i>							
F_{AMF}	2.0	9.1**	0.2	1.6	0.3	0.1	0.9
M_{AMF}	1.1	6.0*	1.0	0.8	1.1	0.3	0.6
A_{AMF}	1.1	6.3*	0.9	0.8	1.2	0.3	0.4
Shoot mass	8.1**	1.8	1.3	0.7	2.9	3.7	2.4
PI_{ABS}	10.2***	1.0	0.9	0.1	0.1	0.0	2.0
Element concentrations in shoots							
N	5.7**	0.0	1.3	0.0	4.8*	0.4	0.2
P	5.5**	0.2	2.0	2.6	2.8	0.3	2.7
Ca	0.8	0.6	1.2	0.0	3.4*	0.1	0.3
Mg	0.2	0.7	1.3	1.9	2.3	7.1*	0.2
Cu	1.4	2.5	9.3***	0.0	5.7**	0.0	0.1
Zn	0.4	0.6	2.4	1.1	0.9	0.4	0.6
<i>Trifolium repens</i>							
F_{AMF}	0.1	3.7	0.3	2.0	1.3	0.6	0.5
M_{AMF}	0.1	3.5	0.4	2.3	2.0	1.0	0.6
A_{AMF}	0.1	2.8	0.4	3.1	2.6	1.7	0.3
Shoot mass	1.5	1.4	0.8	0.0	1.3	0.5	2.5
PI_{ABS}	2.2	0.4	2.5	6.8*	1.3	2.0	0.9
Element concentrations in shoots							
N	2.0	0.0	0.1	0.3	0.5	2.5	0.5
P	0.9	1.3	1.4	7.2*	0.7	3.9	0.4
Ca	0.1	7.9**	2.5	0.1	3.2	0.0	0.5
Mg	1.0	13.3***	1.3	0.7	1.0	0.1	3.1
Cu	0.4	0.0	4.1*	4.4*	1.0	0.3	0.4
Zn	2.2	8.8**	4.2*	1.2	1.6	1.2	1.8

The asterisks indicate statistically significant effects (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$)

AMF spore number, species richness, and composition

In total, the spores of 29 AMF taxa were isolated. The spores of *Septoglomus constrictum*, *Scutellospora dipurpurens*, *Claroideoglossum claroideum*, *Funneliformis mosseae*, and

Rhizoglossum fasciculatum were most frequent, being found in 65, 25, 21, 21, and 15 plots, respectively (Table 3).

Generally, the number of AMF spores and the number of AMF species (species richness) decreased as a result of plant invasion (Table 2, Fig. 1d, e). This effect was particularly

Fig. 1 Means and standard errors ($N = 8$) of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) parameters of the plots with native and invasive plants grouped according to the invader species (*Reynoutria japonica*, *Rudbeckia laciniata*, and *Solidago gigantea*) and the type of habitat (O and V—outside and within river valleys, respectively). **a** Mycorrhizal frequency (F_{AMF}). **b** The frequency of occurrence in roots of dark septate endophytes (F_{DSE}). **c** The concentration of phospholipid fatty acid (PLFA) 16:1 ω 5 AMF marker in soils. **d** The number of AMF spores in 50 g of fresh soil. **e** The number of AMF species (species richness). Inequality expressions in the graphs show significant effects related to the invasion. If the effect of invasion interacted with another effect, contrast analysis was used to compare the native (N) and invasive (I) plant plots separately for each level of a given effect. The involved levels were indicated by subscripted abbreviations: Rj, Rl, and Sg for *R. japonica*, *R. laciniata*, and *S. gigantea*, respectively. The asterisks show the statistical significance of differences between N and I (** $p < 0.01$, *** $p < 0.001$). See Table 2 for the significance of other effects

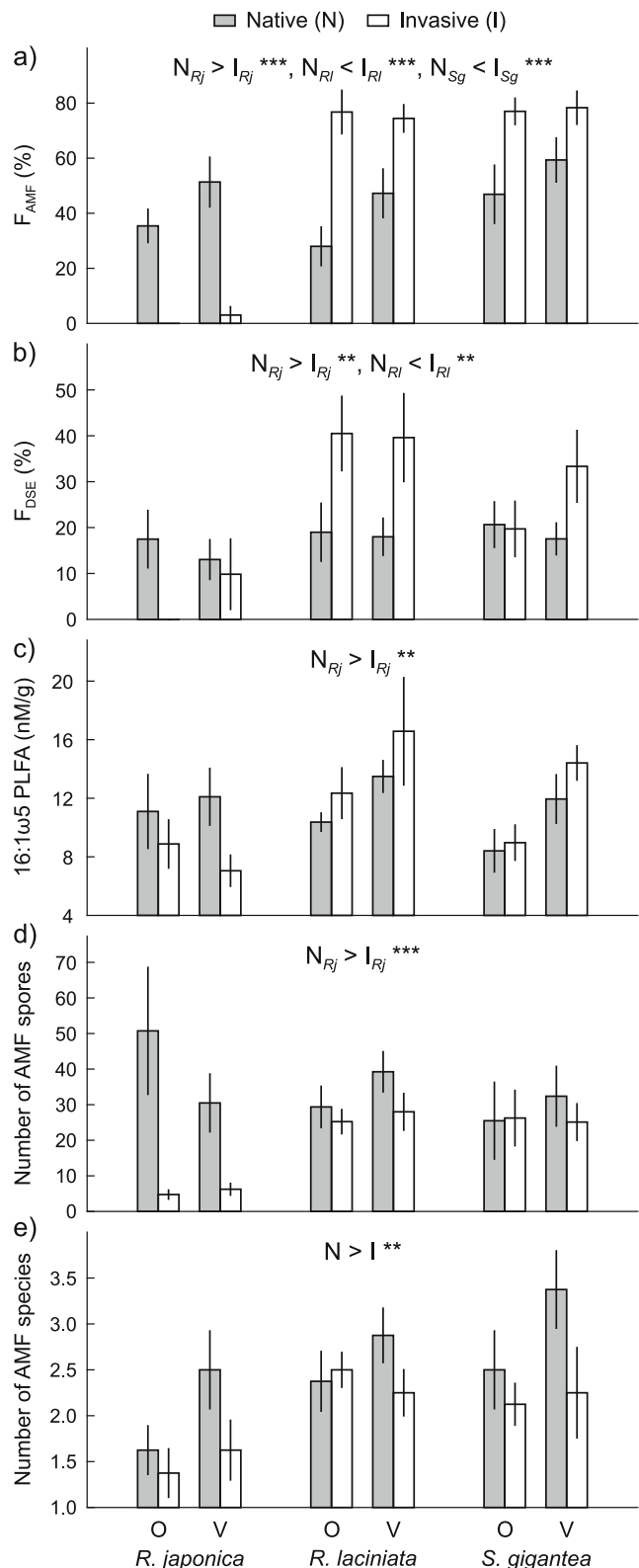
strong in the case of *R. japonica* invasion, which reduced the number of AMF spores much more than the other invaders (significant site \times invasion interaction; Fig. 1d). As regards the species composition, represented by DCA 1 and DCA 2 sample scores, it was insensitive to plant invasion (Table 2).

The AMF species richness associated with the *R. japonica* sites was lower than that associated with the *R. laciniata* and *S. gigantea* sites, as indicated by a significant effect of site (Table 2). Since this difference concerns both the plots infested by alien plants and the plots with native vegetation, variation in AMF species richness among the sites of three invasive plant species thereby suggests that *R. japonica* colonizes sites characterized by lower AMF species richness. A significant effect of site was found also for the AMF species composition; the *R. japonica* sites (including both invader and native vegetation plots) differed from the sites of the other two species in terms of the position in the DCA 2 gradient (Table 2, Fig. 2).

AMF colonization and performance of native plants in soils from under invader and native vegetation

AMF colonization

The AMF colonization of *P. lanceolata* only depended on the type of habitat (Table 2). Plants growing in the soil collected within the river valleys were characterized by higher values of mycorrhizal parameters (F_{AMF} , M_{AMF} , and A_{AMF}) than those growing in the soil from outside the river valleys. These parameters strongly correlated with each other (Pearson's r was from 0.77 to nearly 1), and thus, only the mycorrhizal frequency (F_{AMF}) is presented (Fig. 3a). Regarding the AMF colonization of *T. repens*, none of the effects studied was statistically significant.



Growth response

There were no visual differences in the condition of the plants between all treatments during the whole period of the

Table 3 The occurrence of arbuscular mycorrhizal fungi (AMF) taxa in invader (*I*) and native (*N*) vegetation plots in habitats within and outside river valleys

Species	Frequency ^a	<i>Reynoutria japonica</i>				<i>Rudbeckia laciniata</i>				<i>Solidago gigantea</i>			
		Outside-valley		Valley		Outside-valley		Valley		Outside-valley		Valley	
		N	I	N	I	N	I	N	I	N	I	N	I
<i>Acaulospora capsicula</i>	2							1			1		
<i>Acaulospora cavernata</i>	10					2	3	2	2		1		
<i>Acaulospora paulinae</i>	6					1			1	1	1	1	1
<i>Acaulospora polonica</i>	1						1						
<i>Acaulospora rugosa</i>	1								1				
<i>Acaulospora</i> sp.	1				1								
<i>Ambispora gerdemannii</i>	1												1
<i>Cetraspora pellucida</i>	7	1		1			1	1				1	2
<i>Claroideoglossum claroideum</i>	21			4	1	1	3	2	2	2	2	3	1
<i>Claroideoglossum drummondii</i>	5		1			1				1	1		1
<i>Claroideoglossum etunicatum</i>	2	2											
<i>Entrophospora infrequens</i>	1									1			
<i>Funneliformis caledonius</i>	2				1						1		
<i>Funneliformis geosporus</i>	2							1	1				
<i>Funneliformis mosseae</i>	21	1		2	2	1	1	2	2	3	1	3	3
<i>Funneliformis</i> sp.	2			1	1								
<i>Gigaspora margarita</i>	3			1						1		1	
<i>Glomus aureum</i>	1											1	
<i>Glomus macrocarpum</i>	1							1					
<i>Glomus rubiforme</i>	1	1											
<i>Glomus</i> sp. 1	9	1	3			2		1				1	1
<i>Glomus</i> sp. 2	1								1				
<i>Pacispora franciscana</i>	5			1		1	1	1				1	
<i>Pacispora scintillans</i>	4	1		1				1					1
<i>Paraglossum majewskii</i>	3			1	1							1	
<i>Rhizoglossum fasciculatum</i>	15			1		3	1	1	1	4	1	3	
<i>Scutellospora dipurpurea</i>	25	1	1	1		5	3	4		2	3	3	2
<i>Scutellospora</i> sp.	1								1				
<i>Septoglossum constrictum</i>	65	5	6	6	6	2	6	5	6	5	5	7	6
Total		13	11	20	13	19	20	23	18	20	17	27	18

^a The number of plots in which particular AMF taxa were found

experiment. Also, there were no differences in shoot mass at the end of the experiment, with one exception: *P. lanceolata* individuals growing in the soil taken from *R. laciniata* sites

(including both invader and native vegetation plots) had a higher shoot mass than the individuals growing in other soils (significant effect of site; Table 2, Fig. 3b).

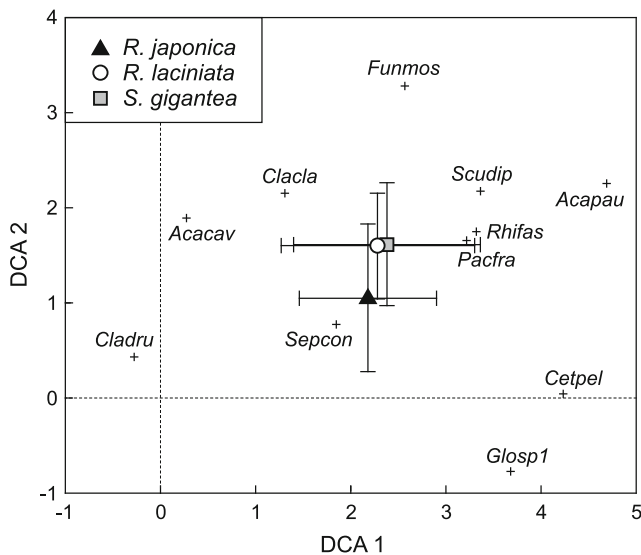


Fig. 2 The result of detrended correspondence analysis performed on the 11 most frequent arbuscular mycorrhizal fungi (AMF) species and 91 samples (5 samples were excluded as they did not contain any species). The first and the second axes explain 15.4 and 9.7 % of the variation in the AMF species composition. Symbols represent the average positions (with standard deviations) of sample plots, grouped according to the invader species, on the DCA 1 and DCA 2 axes. Species are abbreviated by the first three letters of their genus and species names (see Table 3 for the full names of species)

Photosynthetic performance

Photosynthetic performance of *P. lanceolata*, as expressed in PI_{ABS} , varied in a similar manner as the *P. lanceolata* shoot mass (significant effect of site; Table 2): it was higher for plants growing in the soil from *R. laciniata* sites than plants growing in the soil from other sites (Fig. 3c). In the case of *T. repens*, PI_{ABS} was related to invasion (Table 2): the values of this parameter were lower for plants growing in the soil from under invaders (irrespective of the invader species) than those growing in the soil from under native vegetation (Fig. 3c).

Element concentrations in shoots

Invasions significantly affected the concentration of some elements in the *P. lanceolata* and *T. repens* shoots (Table 2). *Plantago lanceolata* growing in the soil influenced by *R. japonica* accumulated less N and Cu in shoots than that growing in the soil from under native vegetation (significant site \times invasion interaction; Fig. 4a, e). Although significant site \times invasion interaction was found in the case of Ca (Table 2), the contrast analysis did not reveal any significant differences between the treatments. Individuals of *P. lanceolata* growing in the soil influenced by *S. gigantea* accumulated more Cu than these in the soil from under native vegetation. The concentration of Mg in *P. lanceolata* shoots was also affected by invasion, but the effect depended on the habitat type: it was visible in sites outside the river valleys

(Fig. 4d). In the case of *T. repens*, only the concentration of P and Cu was influenced by invasion. The plants growing in the soil from the invader plots accumulated more P and less Cu than the plants growing in the soil from the plots of native vegetation (Fig. 4b, e).

The shoot concentration of some elements was also shaped by the effect of habitat, the effect of site, or the interaction of both (Table 2). For example, the concentration of P in the *P. lanceolata* shoots depended on the site: the plants growing in the soil collected in the *S. gigantea* sites accumulated more P than the plants growing in the soil from *R. laciniata* sites (Fig. 4b). The concentration of Cu in the *P. lanceolata* shoots was particularly high for the plants growing in the soil from *R. japonica* sites, but only those located outside the river valleys (significant site \times habitat interaction; Fig. 4e). The concentration of Ca and Mg in the *T. repens* shoots was higher for the plants growing in the soil taken from river valleys than those growing in the soil from outside river valleys (Fig. 4c, d). The opposite situation was found for the concentration of Zn in the *T. repens* shoots, but it concerned only the soil from the *R. japonica* sites (significant site \times habitat interaction; Fig. 4f).

Discussion

Invasive plants may impact on the abundance, activity, and composition of mycorrhizal community in different ways. Invaders that are non-mycorrhizal or have low symbiotic dependency can inhibit AMF, therefore reducing the competitiveness of native plants that rely on AMF. In contrast, invaders that are more dependent on mycorrhizal association may modify the AMF abundance and species richness and as a consequence, receive greater benefits from it than native plant species (Shah et al. 2009).

In line with our expectations, decreased AMF abundance and species richness were observed under the non-mycorrhizal *R. japonica*. Tanner and Gange (2013) found a reduction of mycorrhizal colonization rates of native plants grown in soils infested by this species. It was also showed that under non-mycorrhizal *Alliaria petiolata* (Brassicaceae), the invader of North American forests, both AMF abundance and diversity were decreased (Callaway et al. 2008). Several mechanisms may be involved in the impact of invasive plants on AMF community. First, the reason for this can be simply that the formation of monospecific patches by a non-mycorrhizal plant, such as *R. japonica*, depletes AMF by starving them of a host and subsequent organic C inputs (Tanner and Gange 2013). Although AMF can survive for some periods without a host, their growth and proliferation would be decreased in the absence of mycorrhizal plant species (Tanner and Gange 2013; Zubek et al. 2013). Second, it was found that *A. petiolata* suppresses native plant growth by

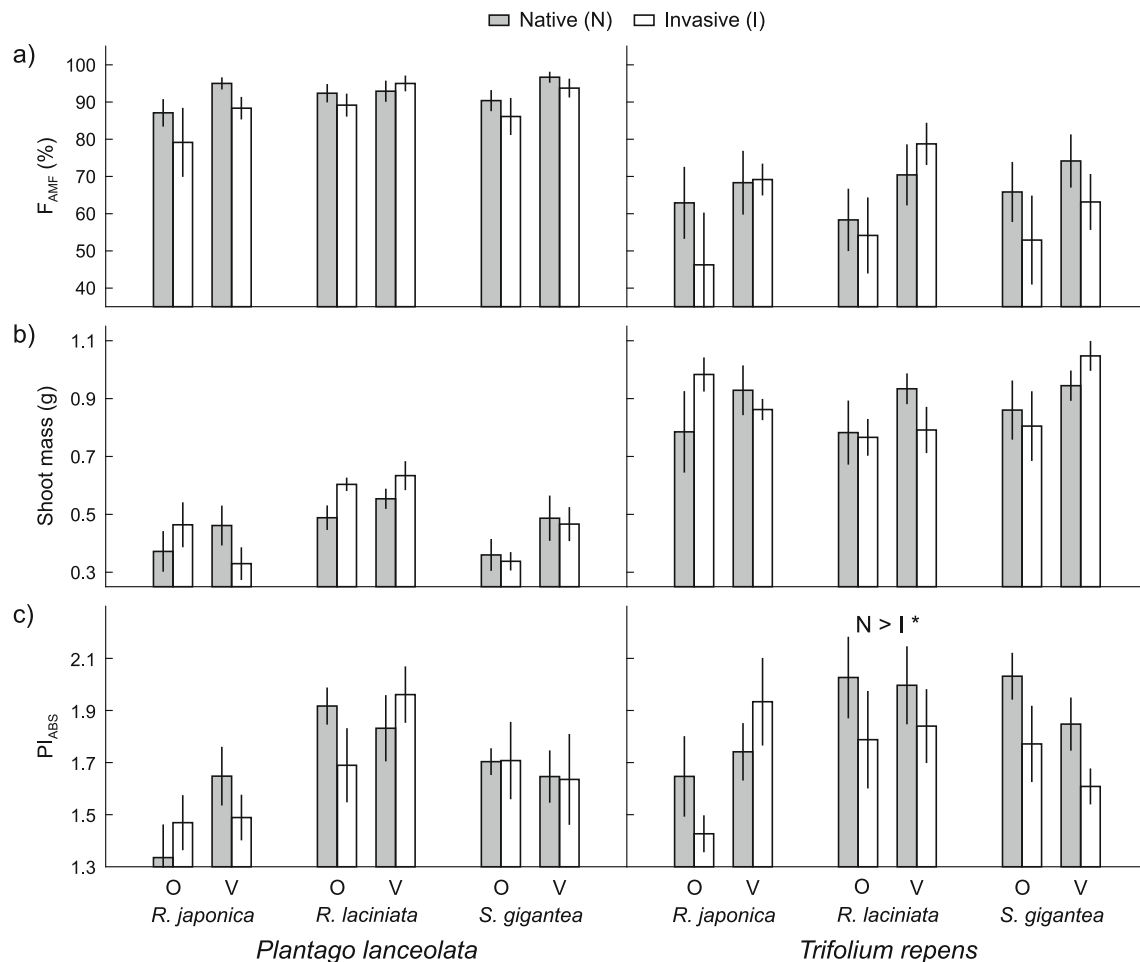


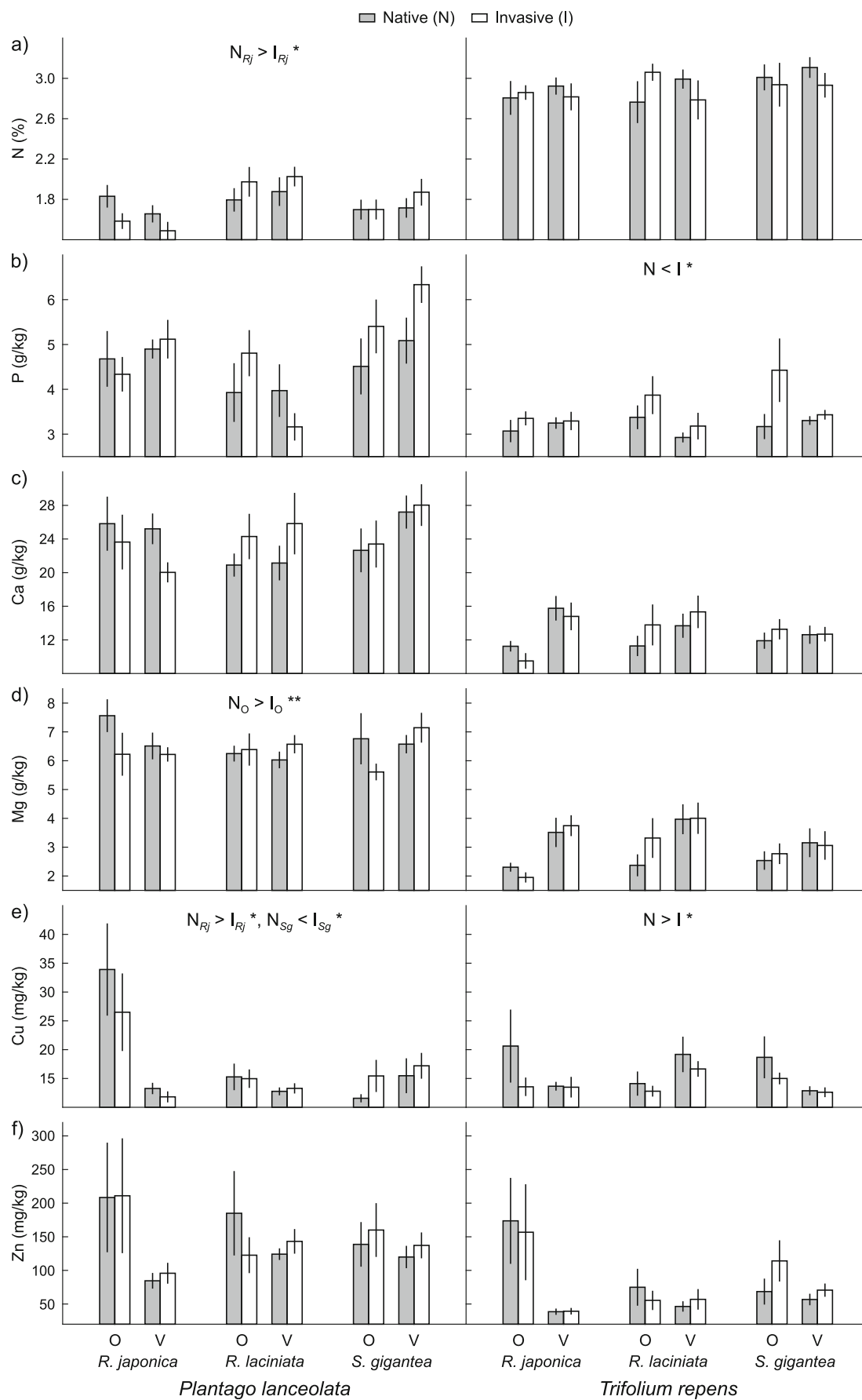
Fig. 3 Means and standard errors ($N=8$) of **a** the mycorrhizal frequency (F_{AMF}), **b** shoot dry mass, and **c** photosynthetic performance index (PI_{ABS}) of native plant species (*Plantago lanceolata*, *Trifolium repens*) grown in soils from adjacent plots with native (N) and invasive (I) plants grouped according to the invader species (*Reynoutria japonica*,

Rudbeckia laciniata, and *Solidago gigantea*) and the type of habitat (O and V—outside and within river valleys, respectively). Inequality expression in the graph shows significant effects related to the invasion. The asterisk shows the statistical significance of differences between N and I ($p < 0.05$). See Table 2 for the significance of other effects

disrupting their mutualistic associations by producing secondary metabolites that directly limit AMF development. Moreover, this plant had no effect on AMF abundance and diversity in soils of its natural range in Europe. Thus, it has been hypothesized that it produces metabolites that are novel to North America and to which North American AMF are not adapted (Stinson et al. 2006; Callaway et al. 2008). Yuan et al. (2014) provided indirect evidence that the secondary metabolites of a mycorrhizal invader in China, *Solidago canadensis*, selectively impact on the composition of AMF community by enhancing the most beneficial AMF and inhibiting less favorable ones. Third, plant invasions through their influence on soil properties may indirectly affect composition of AMF communities that in turn can translate into the success of invasive species (Shah et al. 2009). As revealed by our investigation, the invasions of plants under study significantly changed some chemical soil properties, e.g., decreased P content and increased C:P ratio, whereas *R. japonica* increased

$N-NO_3^-$ concentration (Stefanowicz et al., unpublished). The aforementioned and also other factors could be responsible for the modification of AMF communities in the invaded sites.

Fig. 4 Means and standard errors ($N=8$) of the concentration of elements (N, P, Ca, Mg, Cu, Zn) in shoots of native plant species (*Plantago lanceolata*, *Trifolium repens*) grown in soils from adjacent plots with native and invasive plants grouped according to the invader species (*Reynoutria japonica*, *Rudbeckia laciniata*, and *Solidago gigantea*) and the type of habitat (O and V—outside and within river valleys, respectively). Inequality expressions in the graphs show significant effects related to the invasion. If the effect of invasion interacted with another effect, contrast analysis was used to compare the native (N) and invasive (I) plant plots separately for each level of a given effect. The involved levels were indicated by subscripted abbreviations: *Rj* and *Sg* for *R. japonica* and *S. gigantea*, respectively; O for habitats outside river valleys. The asterisks show the statistical significance of differences between N and I (** $p < 0.01$, * $p < 0.05$). See Table 2 for the significance of other effects



Higher mycorrhizal colonization rates were found in the case of *R. laciniata* and *S. gigantea* plots than those of native vegetation. However, decreased AMF species richness under both invaders was observed. The effects of invasive plants from Asteraceae on AMF were investigated in Asia and North America. A study by Shah et al. (2010) showed that the invasions of mycorrhizal *Anthemis cotula* and *Conyza canadensis* in India decreased species diversity but increased the number of AMF spores in comparison to native vegetation. *Ageratina adenophora* increased the abundance of AMF in soils, and this was hypothesized to be responsible for its invasion process in China (Hong-bang et al. 2007). Inconsistent results were obtained in the case of *Centaurea stoebe* (syn. *C. maculosa*) in the USA. Mummey and Rillig (2006) observed a reduction of AMF diversity in response to *C. stoebe* invasion, whereas Lekberg et al. (2013) showed that this invader supported a higher AMF abundance and diversity than multi-species native plant communities. Although most AMF associate with a wide range of hosts, the selectivity of some plant species for particular fungal symbionts exists (Helgason et al. 2002; Holland et al. 2014; Taylor et al. 2015). The selectivity of an invasive plant for most efficient fungal partners may change AMF species richness and composition to facilitate invader and reduce native plant performance. Zhang et al. (2010) and Yang et al. (2014) found that *S. canadensis* in China changed the dominant species in AMF community due to host-AMF preference. This alteration generated positive feedback to the invader while reducing preferred mutualisms and competitiveness of resident plants. Further studies are needed to elucidate if the alterations in AMF species richness can benefit *R. laciniata* and *S. gigantea* performance in comparison to native plants.

There are over 130 alien plant species in the flora of Poland that belong to Brassicaceae, Caryophyllaceae, Chenopodiaceae, and Polygonaceae (Tokarska-Guzik et al. 2012). The representatives of these families are mostly non-mycorrhizal (Wang and Qiu 2006; Smith and Read 2008). Their impact should be evaluated first as they may cause the most serious reductions in AMF abundance and species richness. However, as revealed by the aforementioned studies and our observation, even the presence of invaders from Asteraceae, AMF-host family, can have a negative effect on AMF species richness.

We showed that *R. japonica* did not eliminate AMF from invaded soils as both some levels of AMF-PLFA marker and spore presence were detected under this species, as well as native plants grown subsequently in these soils had AMF colonization. This finding is in line with the study by Tanner and Gange (2013).

The persistence of AMF under *R. japonica* could stem from few accompanying mycorrhizal species that were found in the case of several plots, the transport of AMF propagules from adjacent areas or their long-term survival from the period before the invasion.

Although in most AMF a large proportion of total fatty acids is found as 16:1 ω 5, this compound is also a constituent in some bacterial genera (Olsson 1999). Thus, our result concerning AMF abundance based on the concentration of this marker could be affected by the amount of bacteria in the soils. Nevertheless, the tendencies in AMF abundance revealed by PLFA analysis were supported by the trends in mycorrhizal colonization rates or spore counts.

During the examination of field-collected roots, we also found the mycelia of dark septate endophytes. The dark septate endophyte root colonization was reduced in *R. japonica* and increased in *R. laciniata* plots in relation to that recorded in the adjacent native vegetation. This pattern reflects the general fungal abundance in soils under study as revealed by PLFA analysis (Stefanowicz et al. 2016). Knapp et al. (2012) found that the same dark septate endophyte isolates were detected from the roots of both alien and native plants in Hungary, supporting the hypothesis that this group of fungi has a wide host range. However, their effect on alien plants and potential role in their invasions remain to be investigated.

It is not clear whether the alterations in AMF abundance and species richness found in our study is exclusive to the habit of the invader species, or if similar patterns can arise by any plant attaining local dominance (Mummey and Rillig 2006; Jandová et al. 2014). As the study was based on an observational approach, we cannot distinguish whether the modifications were due to invasions of the species, or rather due to the loss of native species (Jandová et al. 2014). This is presently investigated in a pot experiment that includes studying the impact of both native and invasive plants on soil chemical and microbiological properties.

We collected samples from paired plots, which were established in adjacent patches of invasive and native plants. Such a sampling scheme did not exclude with certainty the possibility of differences in soil properties between these plots existing prior to invasion events. This is a typical problem in all space-for-time-substitution studies (Dostál et al. 2013). However, we did our best to control for such differences. The plots were established as close as possible to each other, in flat terrain and far from shrubs or trees.

As we used the classical method of AMF species identification and sampled once a year, the results on AMF diversity can be underestimated. We could have overlooked AMF species that produce spores rarely or seasonally (Njeru et al. 2015). Moreover, by sampling to the depth of 20 cm,

some part of spore diversity that exclusively occurred in the subsoils might have been missed (Oehl et al. 2005). This remains to be studied using molecular tools at different seasons and depths of a soil profile.

In our research, the effect of invasion on AMF abundance and species richness did not generally vary between habitats located within and outside river valleys. This pattern reflects the lack of differences in the impact of *R. japonica*, *R. laciniata*, and *S. gigantea* invasions on physicochemical and microbiological soil properties between these locations (Stefanowicz et al. 2016). Fumanal et al. (2006) found that individual plant root colonization of the European invader *Ambrosia artemisiifolia* was significantly different between habitats. There was a general tendency for AMF colonization of *A. artemisiifolia* to increase from cultivated to noncultivated lands in France, which may be related to differences in the physicochemical properties of soils or to discontinuation of agricultural practices, such as fungicide applications or tillage (Fumanal et al. 2006). Chmura and Gucwa-Przepióra (2012) also found differences in the mycorrhizal colonization rates of invasive *Impatiens parviflora* between forest types in Poland that were due to differences in soil chemical properties.

We showed that *R. japonica* encroached on sites of low AMF species richness. This is in accordance with our observations that the sites invaded by this species are in general characterized by low microbiological quality (Stefanowicz et al. 2016). Though *R. japonica* preferentially invades areas with low AMF species richness, it also appears to further reduce AMF species richness within these sites.

The decreased emergence, survival, growth, or nutrient uptake of several native plant species were found on soils overgrown by alien plants in comparison to soils from under native vegetation in North America (Stinson et al. 2006; Callaway et al. 2008; Vogelsang and Bever 2009; Wilson et al. 2012) and Asia (Zhang et al. 2010). The decreased levels of AMF colonization of *P. lanceolata*, *Trifolium pratense*, and *Lotus corniculatus* due to the invasion by *R. japonica* and *Impatiens glandulifera* were also found by Tanner and Gange (2013) in the UK. The percentage colonization of AMF and shoot mass were positively correlated for *P. lanceolata* and *L. corniculatus*, suggesting that their performance can be influenced by depleted levels of AMF. In our study, the invasions affected the performance of native plants. However, the directions and magnitude of their response depended on both species identity and the mycorrhizal status of invaders. *Plantago lanceolata* grown in soils from under *R. japonica* accumulated less N, Mg, and Cu in shoots than the ones grown in soils from adjacent native vegetation. For *Trifolium repens*, no specific effects of a particular invasive plant species were found. However, the individuals of *T. repens* grown in invaded soils had lower photosynthetic performance and shoot Cu concentration. We also found shifts in the concentration of two elements in the native plants due to soil

feedbacks by the invaders. As no significant differences were found in the mycorrhizal colonization rates, it is not clear whether these responses were related to the alterations in AMF abundance and species richness caused by invasions or by other soil biological and physicochemical properties. Different growth response of *T. pratense* and *P. lanceolata* in invaded soils was supposed to be due to the dependency of *T. pratense* on rhizobia symbiosis (Tanner and Gange 2013). The interaction of *T. repens* with bacteria might also be responsible for various responses of two native plant species in our experiment. The lack of differences in mycorrhizal colonization and growth of *P. lanceolata* in our study in comparison to that found by Tanner and Gange (2013) in soils from under *R. japonica* may be due to a variety of environmental factors including differences in soil chemical properties and microbial communities, e.g., AMF species composition, as well as laboratory conditions.

Conclusions

Our investigations included three invasive plant species and two habitat types and used replicate independent sampling over a vast area, thus enabling strong inferences on the effects of their invasions. The impact of *R. laciniata* and *S. gigantea* invasions on AMF communities as well as *R. japonica* on AMF species richness was reported for the first time. The invasion of all plant species influenced AMF abundance or species richness compared to native vegetation, but the extent of changes depended on their mycorrhizal status. The impact of non-mycorrhizal *R. japonica* was the most pronounced as showed by the reduced AMF abundance and species richness. The effects of invasions did not generally differ between valley and outside-valley habitats, probably because they were largely similar in initial soil characteristics as well as native plant species richness and plant cover (Stefanowicz et al. 2016). The photosynthetic performance and the concentrations of some elements in the shoots of *P. lanceolata* or *T. repens* were affected by invasions, but the directions and magnitude of their responses depended on both species identity and the mycorrhizal status of invaders.

The present contribution may have applications in conservation biology for habitat restoration after alien invasion through soil-based management. We agree with the suggestions that after removal of invasive plants, the plots should be restored with native plant species at levels of diversity similar to that of the surrounding vegetation in order to promote AMF proliferation and diversity (Zubek 2012; Tanner and Gange 2013). It is also worth noting that manual and mechanical removal of invaders may intensify the disturbance of the microbial community by further disrupting and depleting the AMF network (Tanner and Gange 2013; Lankau et al. 2014).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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